Anti-inflammatory efficiency of erythropoietin in sciatic nerve damage

The effects of EPO on sciatic nerve

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Abstract

Aim: Peripheral nerve injuries are the major health problem in the world. Several neurotrophic agents have been evaluated for their efficacy in nerve injury. We aimed to compare the effects of erythropoietin (EPO), known to have neuroprotective, neuroregenerative and anti-inflammatory properties like gabapentin, on the sciatic nerve in experimental crush injury to the sciatic nerve in rats. Material and Method: We classified four groups for this study. Aneurysm clips were used for the sciatic nerve crush injury for trauma groups. Sciatic nerve and blood samples were taken for the experimental procedures. ELISA method was used to evaluate the tissue. Results: Average values of IL-1β and TNF-α levels of the groups and the relationship between the groups are presented. After EPO and gabapentin treatment, IL-1β levels were significantly lower in the trauma group. TNF-α levels were statistically significantly higher in the trauma group than in other groups. According to the EPO and gabapentin treatment, TNF-α levels were significantly lower than those in the trauma group. Discussion: In this study, the effects of EPO and gabapentin on the rat sciatic nerve injury were examined by biochemical methods. EPO is a drug that has an advantage of the treatment of anemia and used for the nerve damage in the experimental studies. We suggest that the EPO has beneficial effects on the trauma models with the blood loss and the nerve damage.

Keywords

Sciatic Nerve; Erythropoietin; Gabapentin; Cytokines

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Introduction

Peripheral nerve injuries are frequently encountered in clinical practice. Its yearly incidence is 1/1000 [1]. Significant histopathological changes occur after damage occurring due to post-traumatic nerve stretching, crushing and cutting. The axonal structure is completely damaged by these histopathological changes, and the impulse transfer is destroyed in the first 48–96 hours following nerve injury [2]. The sciatic nerve, dorsal root ganglion, red blood cell and surrounding muscle tissue are affected by the damage [3]. Impulse cannot be transmitted to the muscle or sensory organs stimulated by the damaged nerve cell, and a neurological dysfunction develops depending on the affected area [4].Disruption of blood flow due to crushing over the nerve and the presence of reperfusion after the restoration of the damage causes the formation of acute phase reactants (cytokines such as TNF-α and IL-1β) and free oxygen radicals. Furthermore, these cytokines that form in the neuro-inflammation cascade intensify the damage and result in myelin phagocytosis and the prevention of regeneration in damaged nerve cell [5,6].Although the efficiency of neurotrophic drugs, steroids, hormones, low dose radiation and different chemical materials in increasing nerve regeneration is reported today, and the highest level is technically reached in nerve repair with the development of microsurgical techniques, a total recovery cannot be provided in motor and sensory terms in nerve regeneration [1-3,7,8]. The peripheral nervous system is more successful in axonal regeneration as inhibitor myelin proteins are less dominant and Schwann cells are more active in distal. Therefore, agents considered to increase nerve regeneration are frequently used in experimental studies where peripheral nerve damage is formed [9, 10]. Although medications, such as methylprednisolone and gabapentin, are proven to be effective on post-traumatic nerve injuries (spinal cord traumas and peripheral nerve injuries, etc.) and are considered to be the reference point in the studies made, they are drugs with a difficult clinical application due to their present side effects [8].

Erythropoietin (EPO) is defined as a hematopoietic growth factor with a molecular weight of 30.400 Da and is composed of 165 amino glycoproteins [9,10]. EPO is secreted in renal tubular cells in adults and through hepatic cells in the fetus [10]. It is a drug with low side effect profile and is used in anemia treatment [11]. In recent years, it has drawn attention in experimental studies on nerve damages with its proven anti-inflammatory, neuroprotective and neuroregenerative effects with minimum side effects [9].Our aim in this study was to show the effects of EPO which is known for its neuroprotective, neuroregenerative and anti-inflammatory characteristics in experimental crush type of sciatic nerve damage in rats and to compare it with gabapentin which is known for its effects on acute phase reactants.

Material and Method

Taking previous experimental studies on the subject as a reference, four groups (n=10) were formed using 40 Spraque-Dawley female rats.Sciatic nerve damage was not done on the rats in group 1. Aneurysm clip was held over the sciatic nerve for 90 seconds in the rats in Group 2 and damage was formed. 20mg/kg EPO was applied intraperitoneally for treatment after forming damage with the same method in the rats in Group 3 and 90 mg/kg gabapentin was applied intraperitoneally after damage formation in the rats in Group 4.

At the 96th hour after the treatment, the rats were re-operated, and their sciatic nerve was excised for examination (Figure 1). After the procedure, blood samples were taken from the heart for analysis and the rats were sacrificed after emptying cardiac blood.

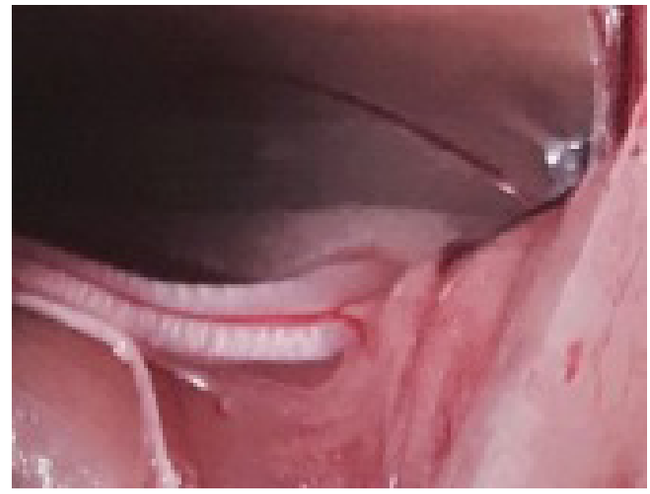

Figure 1. At the 96th hour after the treatment, the rats were re-operated and their sciatic nerve was excised

In order to evaluate the inflammatory response occurring in the environment due to ischemic reperfusion after peripheral nerve damage and EPO effects on this response, TNF α (Serum, Tissue Rat TNF-α ELISA Kit, Sandwich ELISA, LifeSpan BioSciences, USA) and IL-1β (Serum, Tissue Rat IL-1β ELISA Kit, Sandwich ELISA, LifeSpan BioSciences, USA) levels were measured on the serum and sciatic nerve tissue was taken out.Tissue samples taken from the subjects for biochemical analysis after peripheral nerve damage were homogenized in 0.9 NaCl with Janke-Kunke branded ultraturrax T-25 model tissue homogenizer device (IKA®-Werke GmbH & Co. KG., Staufen, Breisgau, Germany). Homogenized samples were centrifuged with Hettick-Universal 320-R brand centrifuge device (Hettich Lab Technology, Tuttlingen, Germany) at 5000 RPM, +4 degrees for 30 minutes. IL-1β (rat IL-1β ELISA kit, Sandwich ELISA, LifeSpan BioSciences, USA) and TNF-α (rat TNF-α ELISA kit, Sandwich ELISA, LifeSpan BioSciences, USA) levels which are acute phase reactants in the acquired supernatants were measured with ELISA method. Measured activities were calculated by proportioning tissue per gram protein with Lowry method. Also, IL-1β (rat IL-1β ELISA kit, Sandwich ELISA, LifeSpan BioSciences, USA) and TNF-α (rat TNF-α ELISA kit, Sandwich ELISA, LifeSpan BioSciences, USA) levels were measured in the blood samples collected from the animals at the 96th hour.

Data analysis was made with IBM SPSS 21 package program. Quantitative variable values were shown as mean±standard deviation or median (Q1-Q3). Normal distribution accordance of quantitative variables was examined with Shapiro Wilk test. One way analysis of variance was used for groups with normal distribution and Kruskal Wallis test was used for the others. Tukey test was used for non-homogenous variances in the mutual comparison of the groups for the variables with a significant difference in one way analysis of variance and Tamhane

test was used for the others. Dunn's test was used for the mutual comparisons of the variables with a significant difference in Kruskal Wallis test. P <0.05 was accepted as significant.

Results

When he results are evaluated in general, a significant difference is observed in all variables among trauma and control groups. Values in trauma group are significantly higher than the control group.Since Serum IL-1β, Serum TNF-α, Tissue IL-1β variables have a normal distribution among the groups, they were evaluated with One-way analysis of variance. Values were given as mean±standard deviation (Figure 2). Since tissue TNF-α could not be matched, group comparison was made with Kruskal Wallis analysis. Values were given as median (Q1-Q3) (Figure 3).

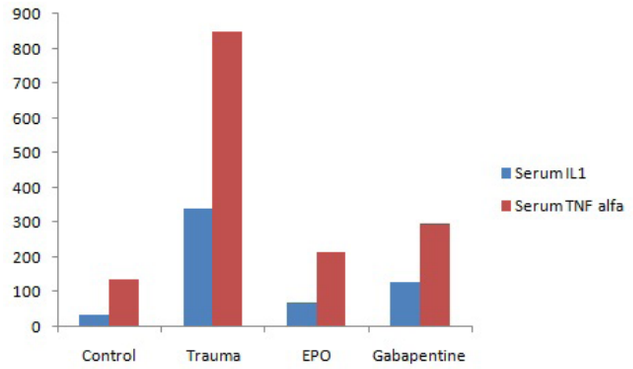

Figure 2.

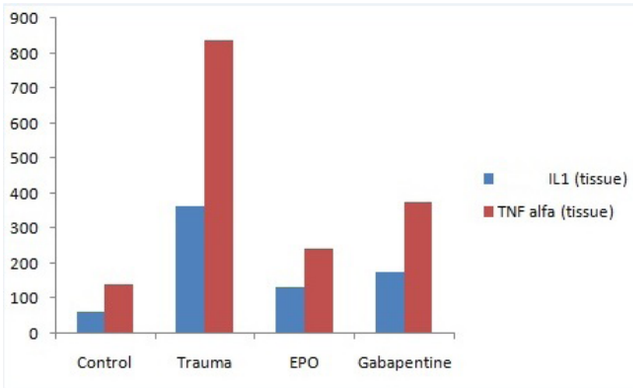

Figure 3.

Since there was no homogenous variance in Serum IL-1β values, a mutual comparison was made with Tamhane test. A significant decrease was observed in serum IL-1β values in EPO, and Gabapentin treatment applied group compared to the trauma group (p=0.004) (Table 1).Tukey test was used for mutual comparison since there was a homogeneous variance in Tissue IL-1β values among the groups. In Tissue IL-1β, there was no difference only among EPO and gabapentin groups (p=0.068). Significant differences compared to the control group were acquired with both drug applications. As a result, IL-1β levels decreased in the tissue in response to EPO and Gabapentin treatment.

IL-1β levels increased after the trauma and decreased significantly both in the tissue and serum in response to the treatment.Tukey test was used for mutual comparison since there

Table 1. Serum IL1 beta comparison with Tamhane test

	Mean Difference	Std. Error	Significance
Control & EPO	-38	9,37	0,004
EPO & Control	+38	9,37	0,004
Gabapentine & EPO	57	11,64	0,001

Table 2. Serum TNF alpha multiple comparison with Tukey

	Mean Difference	Std. Error	Significance
Control & EPO	-78	29,16	0,052
EPO & Control	+78	29,16	0,052
EPO & Gabapentine	-81,5	29,16	0,040
Gabapentine & EPO	+81,5	29,16	0,040

was a homogeneous variance in Serum TNF-α values among the groups. There was no significant difference in the EPO treatment group when compared to the control group. When both treatment groups were compared to trauma group, a significant decrease was observed (p<0.05) (Table 2).

Dunn's test was used for the mutual comparisons of Tissue TNF-α values. While no significant difference was detected in the values in the tissue in the Gabapentin treatment group (p=0.140), a significant difference was detected when EPO treatment group was compared to the trauma group (p=0.02). As a result, while both drug groups were effective in serum levels in the evaluation of TNF-α levels, EPO treatment provided better results in tissue compared to gabapentin.

Discussion

Nerve damage is an irreversible and non-treatable disease frequently studied in recent years. Many studies including stem cell studies have been made for pain and regeneration until today, but none except gabapentin and pregabalin could be used in clinical terms.

With its proven neuroprotective, neuroregenerative and anti-inflammatory efficiency, EPO is a drug which is more commonly used in experimental models in recent years. Wang et al. showed that EPO improved neurological functions in the experimental ischemia model. In another study, Brines et al. showed that the infarct area was smaller in the rat brain with EPO treatment and it had a cell-protecting effect [9]. It was also shown that when EPO is applied in the central nervous system after a stroke, it decreased apoptosis and brain edema [12]. EPO has direct and indirect effects on the nerve cell. It was observed that its neuroprotective efficiency was due to many causes such as the effects on Jack, BCL-2 pathways, and calcium ion flow, changes in glutathione peroxidase activity, normalization of cerebral blood flow, metabolization of free radicals, IL-6 decrease and nitric oxide production increase [13, 14]. It is known that EPO treatment incrases axonal recovery after stroke. This effect is due to EPO's suppressing effect on proteins such as Nago-A and MAG which prevent axonal recovery. It was shown that efficient blood concentrations were reached even in late applications, oligodendrogenesis was stimulated, and white substance damage was decreased in studies where hypoxic-ischemic damage is formed. Successful recovery results were observed in the sixth week after the stroke [15].

In another study examining the effects of EPO on axonal regeneration, it was shown that EPO application was effective on

axonal regeneration in the beginning phase, but current studies are needed for the effective dose and application [10, 16]. Based on the positive neuroprotective and neuroregenerative effects on nerve through local application after optic nerve damage, it is considered that it can reach the effective dose in a shorter time and the treatment efficiency would increase when EPO is applied locally in sciatic nerve damage [13].In another study by Campana et al., it was demonstrated that EPO was released from normal ganglion axon and body in rats and increased Schwann cells after peripheral nerve damage [10,16]. In another study, they showed that it decreased apoptosis in dorsal root cells and provided a protective effect on nerve cell [13].Different proteins are activated through EPO binding to the receptor, and the enzymes stimulating apoptosis are inactivated. The number of EPO-receptors in Schwann cells doesn't increase after peripheral nerve damage, and EPO provides neuronal recovery through endogenous cytokines in the environment [17,18].

In another study, more than 180 recent preclinical studies using EPO for treatment were examined, and it was observed that the basis of 17 studies was the anti-inflammatory effect of EPO. It was shown that EPO decreased inflammation, increased cellular life duration and decreased axonal damage in different experimental models applied in these 17 studies. In head trauma and spinal trauma experiment models, it was shown that brain edema, inflammation, and apoptosis decreased in EPO treatment after trauma, and thus the neurological recovery was higher [19].

IL-1β and TNF-α are important cytokines which are released from glial cells, and they play a key role in chronic pain, memory and damage pathogenesis. It was observed that sciatic nerve damage in the environment increased starting from the fifth hour following nerve damage [5,6,3]. Cytokines such as IL-1β and TNF-α were used to determine the anti-inflammatory effect in different experimental models. In another experimental study where especially the sciatic nerve damage was shown, it was observed that IL-1β levels significantly increased in trauma group compared to the control group [20-22]. In another study prepared based on sciatic nerve damage model, TNF-α, IL-1β, and IL-10 values increased significantly compared to the control group [6,3]. These markers cause neuroinflammation-dependent cascade to start and worsen things after nerve damage. This process is paused through anti-inflammatory activity [22, 5]. Taking experimental studies as a reference, TNF-α and IL-1β were used to evaluate neuroinflammatory cascade in our study, and a significant increase was observed in the trauma group compared to the control group.It cannot be disregarded that myelinization is an important factor in recovery after nerve damage. In the experimental studies made, it was shown that EPO stimulated myelinization quickly after sciatic nerve damage and provided functional recovery [23]. It was also shown that EPO increased white matter myelin thickness, axon diameter, regenerated and myelinated nerve fibers in central nervous system and spinal cord [16,24]. In another study, it was shown that IL-1β activated the macrophages preventing nerve myelinization and thus the anti-inflammatory effect is important for prevention macrophage dysfunction and myelin phagocytosis [21].In a study showing the effect of EPO on peripheral nerve damage, it was declared to be therapeutic which is quickly effective in sudden crash injury model, safe for human use and has anti-inflammatory efficiency [10]. The fast efficiency of the drug used in acute injuries occurring due to trauma is a condition preventing secondary damage and positively affecting nerve recovery. EPO reached the efficient concentration in a short time in our study and provided better treatment results compared to other groups. Therapeutic agent used in acute damages reaching blood concentrations quickly and showing its effect provides an advantage over other drugs. Also, the fact that EPO is a drug used in anemia treatment with its low side effect profile makes us consider that it would also be effective on blood loss occurring with a nerve injury in trauma patients [16].Different medications are being tried for neuropathic pain treatment in peripheral nerve damage. In a study evaluating the effects of tramadol and gabapentin in neuropathic pain treatment and their effects when they are used together, it was shown that the effects of gabapentin and tramadol are dose-dependent and a more effective treatment chance is provided through synergistic effect when they are applied together. When compared to the singular use of these agents, when gabapentin and tramadol effecting from different pain pathways are used together, a higher decrease was observed in IL-1B level which is a proinflammatory cytokine increasing in 7-14 days following the damage and inhibiting in glial functions [25].Despite all current studies made, we think that our available knowledge on the effects of EPO on acute peripheral nerve damage is still not satisfactory and more scientific studies are needed.

Animal studies

All institutional and national guidelines for the care and use of laboratory animals were followed.

Human studies

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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